

WHAT IS CLAIMED IS:

1. A nucleic acid polymerase which has been modified or mutated to increase or enhance fidelity.

2. A nucleic acid polymerase which has been modified or mutated to reduce or eliminate misincorporation of nucleotides during nucleic acid synthesis.

3. The polymerase of claim 1 or 2, wherein said polymerase is a DNA or RNA polymerase.

4. The polymerase of claim 3, wherein said polymerase is mesophilic or thermostable.

5. The polymerase of claim 3, wherein said polymerase is selected from the group consisting of *Tne* DNA polymerase, *Taq* DNA polymerase, *Tma* DNA polymerase, *Tth* DNA polymerase, *Tli* (VENT™) DNA polymerase, *Pfu* DNA polymerase, DEEPVENT™ DNA polymerase, *Pwo* DNA polymerase, *Bst* DNA polymerase, *Bca* DNA polymerase, *Tfl* DNA polymerase, and mutants, variants, fragments, and derivatives thereof.

6. The polymerase of claim 1 or 2, further comprising one or more modifications or mutations to reduce or eliminate one or more activities selected from the group consisting of:

- (a) the 3'→5' exonuclease activity of the polymerase;
- (b) the 5'→3' exonuclease activity of the polymerase; and
- (c) the discriminatory activity against one or more dideoxynucleotides.

7. The polymerase of claim 1 or claim 2, wherein said polymerase is modified or mutated to reduce or eliminate 3'→5' exonuclease activity.

8. The polymerase of claim 1 or claim 2, wherein said polymerase is modified or mutated to reduce or eliminate discriminatory activity.

5 9. The polymerase of claim 1 or claim 2, wherein said polymerase is modified or mutated to reduce or eliminate 5'→3' exonuclease activity.

10. The polymerase of claim 3, wherein said polymerase comprises one or more modifications or mutations in the O-helix of said polymerase.

10 11. The polymerase of claim 10, wherein said O-helix is defined as RXXXXKXXXXFXXXXYX (SEQ ID NO:1), wherein X is any amino acid.

12. The polymerase of claim 11, wherein said mutation or modification is at position R (Arg) of said O-helix.

13. The polymerase of claim 12, wherein said mutation or modification is an amino acid substitution at position R of said O-helix.

15 14. The polymerase of claim 13, wherein R (Arg) is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Try and Val.

15. The polymerase of claim 11, wherein said mutation or modification is at position K (Lys) of said O-helix.

20 16. The polymerase of claim 15, wherein said mutation or modification is an amino acid substitution at position K of said O-helix.

17. The polymerase of claim 16, wherein K (Lys) is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Try and Val.

18. The polymerase of claim 11, wherein said mutations or modifications are at position R (Arg) and at position K (Lys) of said O-helix.

19. The polymerase of claim 18, wherein said mutation or modification are amino acid substitutions at position R and at position K of said O-helix.

20. The polymerase of claim 18, wherein R (Arg) is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Try and Val, and wherein K (Lys) is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Try and Val.

21. A vector comprising a gene encoding the polymerase of any one of claims 1 or 2.

22. The vector of claim 21, wherein said gene is operably linked to a promoter.

23. The vector of claim 22, wherein said promoter is selected from the group consisting of a  $\lambda$ -P<sub>L</sub> promoter, a *tac* promoter, a *trp* promoter, and a *trc* promoter.

24. A host cell comprising the vector of claim 21.

25. A method of producing a polymerase, said method comprising:

- (a) culturing the host cell of claim 24;
- (b) expressing said gene; and
- (c) isolating said polymerase from said host cell.

26. The method of claim 25, wherein said host cell is *E. coli*.

5           27. A method of synthesizing one or more nucleic acid molecules comprising

- (a) mixing one or more nucleic acid templates with one or more of the polymerases of claim 1 or 2 to form a mixture; and
  - (b) incubating said mixture under conditions sufficient to make
- 10 one or more nucleic acid molecules complementary to all or a portion of said one or more templates.

28. The method of claim 27, wherein said mixture further comprises one or more nucleotides selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, ddATP, ddCTP, ddGTP, ddITP,

15 ddTTP, [ $\alpha$ -S]dATP, [ $\alpha$ -S]dTTP, [ $\alpha$ -S]dGTP, and [ $\alpha$ -S]dCTP.

29. The method of claim 28, wherein one or more of said nucleotides are detectably labeled.

30. A method of sequencing one or more DNA molecules, comprising

- (a) hybridizing one or more primers to one or more DNA

20 molecules to be sequenced;

- (b) mixing said one or more DNA molecules to be sequenced with one or more deoxyribonucleoside triphosphates, one or more of the DNA polymerases of claim 1 or claim 2, and one or more nucleic acid synthesis terminating agents to form a mixture;

(c) incubating said mixture under conditions sufficient to produce a random population of synthesized DNA molecules complementary to said one or more DNA molecules to be sequenced, wherein said synthesized DNA molecules are shorter in length than said one or more DNA molecules to be sequenced and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequences of said one or more DNA molecules to be sequenced can be determined.

31. The method of claim 30, wherein said one or more deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, [ $\alpha$ -S]dATP, [ $\alpha$ -S]dTTP, [ $\alpha$ -S]dGTP, and [ $\alpha$ -S]dCTP.

32. The method of claim 30, wherein said nucleic acid synthesis terminating agent is a dideoxynucleoside triphosphate.

33. The method of claim 32, wherein said dideoxynucleoside triphosphate is selected from the group consisting of ddTTP, ddATP, ddGTP, ddITP and ddCTP.

34. The method of claim 30, wherein one or more of said deoxyribonucleoside triphosphates is detectably labeled.

35. The method of claim 32, wherein one or more of said dideoxynucleoside triphosphates is detectably labeled.

36. A method for amplifying a double stranded DNA molecule, comprising

(a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

5 (b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more of the DNA polymerases of claim 1 or claim 2, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

10 (c) denaturing said first and third strand, and said second and fourth strands; and

(d) repeating steps (a) to (c) one or more times.

37. A kit for amplifying, synthesizing, or sequencing a DNA molecule comprising one or more of the polymerases of claim 1 or claim 2.

15 38. The kit of claim 37, further comprising one or more dideoxyribonucleoside triphosphates.

39. The kit of claim 37, further comprising one or more deoxyribonucleoside triphosphates.

20 40. The kit of claim 38, further comprising one or more deoxyribonucleoside triphosphates.

41. A method of preparing one or more cDNA molecules from one or more mRNA templates, comprising

(a) mixing one or more mRNA templates with one or more of the polymerases of claim 1 or claim 2 to form a mixture; and

(b) incubating said mixture under conditions sufficient to synthesize one or more cDNA molecules complementary to all or a portion of said one or more templates.

5           42.     The method of claim 41, further comprising incubating said one or more cDNA molecules under conditions sufficient to make one or more double stranded cDNA molecules.